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ERYTHROCYTE, PLASMA AND BLOOD VOLUME OF HEALTHY YOUNG MEN: RELATIONSHIPS TO BODY SIZE AND AEROBIC FITNESS

BY

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The primary purpose of this study was to develop a normative database for the erythrocyte volume, plasma volume and blood volume of healthy young men. The secondary purposes were to relate these vascular fluid volumes to the person's body size and physical fitness level and to develop regression equations which enable their accurate prediction. Fifty-one male soldiers with a mean age of 22 (range 18 to 35) years and with a maximal aerobic power of 53 (range 42 to 65) ml $O_2 \cdot kg^{-1} \cdot min^{-1}$) had their lean body mass

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and vascular fluid volumes measured. Erythrocyte volume was measured by ^{51}Cr for all subjects; plasma volume was measured by ^{125}I for forty-three subjects and calculated (assumed F-cell of 0.89) from the erythrocyte volume and venous hematocrit for eight subjects. The findings concerning the erythrocyte volume, plasma volume and blood volume of young men are summarized as follows: 1) these vascular fluid volumes are accurately predicted from several indices of body size; 2) lean body mass is the anthropometric index which is most closely correlated to these vascular fluid volumes; 3) aerobic fitness does not influence these vascular fluid volumes in individuals not recently participating in intense physical training; and 4) F-cell ratio is not related to aerobic fitness.

ABSTRACT

The primary purpose of this study was to develop a normative database for the erythrocyte volume, plasma volume and blood volume of healthy young men. The secondary purposes were to relate these vascular fluid volumes to the person's body size and physical fitness level and to develop regression equations which enable their accurate prediction. Fifty-one male soldiers with a mean age of 22 (range 18 to 35) years and with a maximal aerobic power of 53 (range 42 to 65) ml $O_2 \cdot kg^{-1} \cdot min^{-1}$) had their lean body mass and vascular fluid volumes measured. Erythrocyte volume was measured by ^{51}Cr for all subjects; plasma volume was measured by ^{125}I for forty-three subjects and calculated (assumed F-Cell of 0.89) from the erythrocyte volume and venous hematocrit for eight subjects. The findings concerning the erythrocyte volume, plasma volume and blood volume of young men are summarized as follows: 1) these vascular fluid volumes are accurately predicted from several indices of body size; 2) lean body mass is the anthropometric index which is most closely correlated to these vascular fluid volumes; 3) aerobic fitness does not influence these vascular fluid volumes in individuals not recently participating in intense physical training; and 4) F-Cell ratio is not related to aerobic fitness.

Key Words: F-cell ratio, hemtological volumes, lean body mass, normative data, surface area, total blood volume, vascular fluid volumes

INTRODUCTION

Total blood volume is defined as the sum of the erythrocyte volume and plasma volume; and a limited amount of data are readily available concerning these vascular fluid volumes in healthy young men (2). Clinicians and medical scientists need to have an accurate estimate of the erythrocyte, plasma and blood volumes in young men for several reasons. First, young men have a relatively high probability to receive traumatic injuries, suffer hemorrhage and possibly need blood transfusions. This is true in the civilian setting because of their involvement in accidents, whereas, in the military setting these individuals are the most likely to serve in combat and become wounded. Secondly, young men often provide the control group for medical studies that evaluate the effects of factors such as disease, gender, aging, physical activity and environmental adaptations on vascular fluid volumes.

The International Committee for Standardization in Hematology has recommended that erythrocyte volume be measured by the radioactive chromium or technetium methods, and that plasma volume be measured by the radio-iodine labelled albumin method (16). Previous studies which employed these or other methodologies have often not adequately described the age (14,23), health (4,23,29), body sizes (14,25), fitness levels (1,4,8,10,13,21,29,30) of the subject populations, and not performed simultaneous erythrocyte and plasma volume measurements (1,10,13,21,25,29,30). It is known that these vascular fluid volumes should be normalized to body size for comparative purposes (23), however, the best body size index to use is unclear. Furthermore, intense physical training is associated with an elevated plasma volume (7), but it's effect on erythrocyte volume is unclear because most pertinent studies have used the carbon monoxide method (5,9). That method is unsuitable (18) because carbon monoxide's volume of

distribution is to all iron-porphyrin molecules including those in skeletal and heart muscle (31,32); as a result, the volume of distribution is variable between individuals and ranges from 2 to 20 per cent greater than the erythrocyte volume (18,22). The relationships of these vascular fluid volumes to aerobic fitness for subjects not participating in intense physical training are unknown.

Hurley (14) has compiled erythrocyte and plasma volume data from the published literature and developed regression equations to predict these vascular volumes from body surface area. They analyzed data obtained from 481 men who's age ranged from 15 to 90 years and had an undetermined range for body composition and physical fitness. These equations are probably not totally applicable to a stratified population of active, young men. A need exists for a normative database concerning the erythrocyte, plasma and blood volume of healthy men which has been developed from acceptable methodologies for vascular fluid volumes, body size indices (eg. weight, lean body mass, surface area) and aerobic fitness levels (maximal oxygen uptake).

The primary purpose of this study was to develop a normative database for the erythrocyte volume, plasma volume and blood volume of healthy, young men. The secondary purposes were to relate these vascular fluid volumes to the person's body size and physical fitness level and to develop regression equations which enables their accurate prediction.

METHODS

Subjects. Fifty-one healthy male soldiers participated in this investigation. They gave their voluntary and informed consent to participate in this investigation, which had received approval by the appropriate Institutional Review Boards. Investigators adhered to AR 70-25 and

United States Army Research and Development Command Regulation 70-25 on Use of Volunteers in Research. The present subjects were all physically active and participated in their normally required military physical training but none of the subjects were involved in any intense athletic training.

Procedures and measurements. All measurements were obtained in temperate (18-22°C) environments during either the winter or spring months. The subjects had their specific gravity determined by hydrostatic weighing with residual lung volumes measured while they were submerged. The percentage of body fat was calculated from specific gravity by using the equation of Siri. Nude body weights were measured on a K-120 Sauter electronic balance (accuracy 10g) and height was measured with an anthropometer. Lean body mass was calculated as the difference between the body weight and fat weight. Body surface area was calculated from the body weight and height by the DuBois formula.

For thirty subjects, maximal oxygen uptake (aerobic power) was determined from a progressive intensity and continuous effort treadmill protocol (24). The initial treadmill grade was zero, and was increased by 2.5% increments every 1.5 minutes. Each subject's running velocity (2.68 or 3.13 $m \cdot s^{-1}$) was determined from his heart rate response to a ten minute warm-up walk (1.56 m/s at 10%) grade. If the elicited heart rate equaled or was greater than 145 bpm, the 2.68 $m \cdot s^{-1}$ velocity was selected for the maximal test. Established criteria were employed for determination of maximal oxygen uptake for the 2.68 $m \cdot s^{-1}$ and 3.13 $m \cdot s^{-1}$ (20,26) tests. For twenty-one of the subjects, maximal oxygen uptake was determined from a progressive intensity and intermittent effort cycle ergometer protocol (17,33). The subjects initially completed a five minute exercise bout at 180 watts. The heart rate elicited during the final 15 seconds of that bout

was used to predict the maximal oxygen uptake from the nomogram of Astrand (3). The subjects then exercised for five minutes at a power output 30 watts below their predicted maximal oxygen uptake. On subsequent days the subjects performed five minute exercise bouts at 30 watt increments above their previous days bout. Established criteria were employed for determination of maximal aerobic power (17).

Electrocardiograms were obtained with chest electrodes (CM5 placement) and radiotelemetered to an oscilloscope-cardiotachometer unit (Hewlett-Packard). Oxygen uptake was determined by open-circuit spirometry by an automated system (Sensormedics Horizon MMC). Subjects breathed via a two-way valve (Collins two-way J) into the metabolic system. The maximal oxygen uptake values obtained during the cycle ergometer tests were increased by ten-percent to correct them to treadmill values (3,17,19).

Erythrocyte volume and plasma volume were measured by the radioactively labeled chromium-(^{51}Cr) and iodine-labeled (^{125}I) albumin methods, respectively (27). On the measurement day the subjects reported in a post-absorptive state. For the measurement of erythrocyte volume, a 20 ml blood sample was drawn into 3 ml of acid-citrate dextrose and labelled with 11.5 microcurie of ^{51}Cr. For the measurement of plasma volume, a 5 ml aliquot was prepared from a 1.5 microcurie of ^{125}I albumin in 15 ml saline solution. A 10 ml aliquot (5 microcurie) of autologous erythrocytes and the 5 ml aliquot (0.5 microcurie) of albumin solution was then injected into the subject, and blood samples were obtained from the contralateral arm at ten minute intervals over the subsequent thirty minutes. For forty-three subjects the blood volumes represent the sum of the measured erythrocyte and measured plasma volumes. For eight subjects the blood volumes represent the sum of the measured erythrocyte volume and a

calculated plasma volume. The plasma volume was calculated from the measured erythrocyte volume, measured venous hematocrit and an F-cell ratio of 0.89. This F-cell ratio value represents the mean measured value for the forty-three other subjects. The F-cell ratio (6) is the ratio of overall hematocrit (Ho) to the peripheral venous hematocrit (not corrected for trapped plasma). The overall hematocrit was calculated as: $Ho= EV \cdot (EV+PV)^{-1}$

Venous blood samples were collected from a 19 gauge Butterfly needle placed within an antecubital vein. Patency was maintained with saline; the tubing (2 ml of dead space) was flushed with 4 ml of blood before each 9-ml sample was obtained. All blood samples were obtained with the subjects sitting in a supine posture. The subjects maintained this posture for a minimum of thirty minutes prior to any measurements. Hematocrit were obtained from capillary tubes which were centrifuged (IEC MICRO-MB) for 5 minutes at a force of 12,700 G.

The statistical analyses consisted of means, standard deviations and simple regression. Statistical significance was accepted at the $P<0.05$ level.

RESULTS

The ethnic origins for the subject population was forty-one caucasians, seven blacks and three hispanics. Table 1 provides the characteristics of the subject population. The age distribution men was skewed towards the younger subjects as thirty were between 18 to 20 yrs, ten were between 21 to 25 yrs, seven were between 26 to 30 yrs and four were between 31 to 35 yrs. Each of the body size indices (body weight,lean body mass,surface area) were normally distributed throughout a broad range of values. The subjects mean percent body fat was 17±5 and ranged from 7 to 30 percent. Maximal aerobic power was normally distributed throughout

a broad range of values and was not related to the subjects age. The subject's had a venous hematocrit of 41.9±2.3, overall hematocrit of 37.7±2.0 and F-Cell ratio of 0.89±0.04; and none of these variables were related to the subject's age, ethnic group or aerobic power.

The subjects had a mean erythrocyte volume of 1.93±0.26 L that ranged from 1.41 to 2.45 L. The erythrocyte volumes averaged 26 $ml \cdot kg^{-1}$, 1000 $ml \cdot m^{-2}$ and 31 $ml \cdot kg\ LBM^{-1}$ and these values were not related to the subject's ethnic group or age. Maximal aerobic power was related to erythrocyte volume per kilogram body weight ($r=0.58; P<0.05$), but was not related to erythrocyte volume per body surface area ($r=0.20$) or lean body mass ($r=0.20$). Figures 1-3 illustrate the individual data for relationships between erythrocyte volume and the body size indices. Note that the regression equations for erythrocyte volume do not intercept through zero for any of the body size indices, as a result the erythrocyte volume must be estimated from regression equations and not from the ratios of erythrocyte volume to body size indices. The regression equations which employed body weight, surface area and lean body mass accounted for 62%, 64% and 69% of the variance for erythrocyte volume, respectively.

The subjects had a mean plasma volume of 3.25±0.41 L that ranged from 2.70 to 4.28 L. The plasma volumes averaged 43 $ml \cdot kg^{-1}$, 1.685 $ml \cdot m^{-2}$ and 52 $ml \cdot kg\ LBM^{-1}$ and these values were not related to the subject's ethnic group or age. Maximal aerobic power was related to plasma volume per kilogram body weight ($r=0.42; P<0.05$), but was not related to plasma volume per body surface area ($r=0.09$) or lean body mass ($r=0.09$). Figures 1-3 illustrate the individual data for relationships between plasma volume and the body size indices. Note that the regression equations for plasma volume do not intercept through zero for any of the body size indices; as a result the plasma volume must be estimated from regression equations and not from the ratios

of plasma volume to body size indices. The regression equations which employed body weight, surface area and lean body mass accounted for 54%, 59 and 64% of the variance for plasma volume, respectively.

The subjects had a mean blood volume of 5.18±0.65 L that ranged from 4.03 to 6.57 L. The blood volumes averaged 69 $ml \cdot kg^{-1}$ 2685 $ml \cdot m^{-2}$ and 82 $ml \cdot kg\ LBM^{-1}$ and these values were not related to the subject's ethnic group or age. Maximal aerobic power was related to blood volume kilogram body weight ($r=0.52; P<0.05$), but was not related to blood volume per body surface area ($r=0.15$) or lean body mass ($r=0.16$). Figures 1-3 illustrate the individual data for relationships between blood volume and the body size indices. Note that the regression equations for blood volume do not intercept through zero for any of the body size indices, as a result the blood volume must be estimated from regression equations and not from the ratios of blood volume to body size indices. The regression equations which employed body weight, surface area and lean body mass accounted for 64%, 69% and 74% of the variance for blood volume, respectively.

DISCUSSION

This present study is the first to report simultaneously measured the erythrocyte and plasma volumes for a large population of young men as well as to quantitate their lean body mass and physical fitness levels. It is accepted that normative vascular fluid volume data should be corrected for body build (10,30) and/or size (1,13,21,23). In addition, several investigators (11,30) have suggested that normative vascular fluid volume data might need to be correct for physical fitness level; however, no previous database has addressed this conjecture. As a result,

normative vascular fluid volume data are probably only generalizable to populations of similar anthropometric and perhaps aerobic fitness levels. The present population's body sizes and aerobic fitness levels compare favorably to those values recently reported in a survey of U.S. soldiers (28). Vogel and colleagues (28) found that for 1,011 male soldiers, within the age range of this study, that the mean aerobic fitness, body weight and percent fat were 51.1 $ml \cdot kg^{-1} \cdot min^{-1}$ 72.3 kg and 17.0 %, respectively. As a result, the vascular fluid volume data presented in this paper are probably generalizable to men in the U.S. military and other similar groups.

Our data indicate that erythrocyte, plasma and blood volume are all predicted best from the subject's lean body mass (see Figure 3). For each of the vascular fluid volumes, lean body mass accounted for a greater amount of variance than either body surface area or body weight. It should be noted, however, that from our database both body surface area and body weight appear to be adequate predictors of vascular fluid volumes for clinical situations. Previous investigators have reported that vascular fluid volumes are related to surface area and lean body mass (14,23); however, only three studies (1,13,15), concerning young men, have adequately estimated lean body mass as well as either erythrocyte or plasma volume. Allen et al (1) measured lean body mass by hydrostatic weighing and plasma volume by T-1824 dye dilution in Chinese students. We analyzed their data of 41 young men, and found a stronger relationship between plasma volume and lean body mass (r=0.56) than either body surface area (r=0.36) or weight (r=0.26). It should be noted that those subjects had small body masses (mean weight 53.6 kg) compared to their North American counterparts. Huff and Feller (13) measured lean body mass by volume displacement and erythrocyte volume by ^{51}Cr in "normal" men. We analyzed their data, of 33 young men, and found a stronger relationship between erythrocyte volume and

body surface area (r=0.63) than with either body weight (r=0.51) or lean body mass (r=0.44). Hyde and Jones (15) found a stronger relationship between erythrocyte volume (^{51}Cr) and total body water (urea distribution, r=0.98) than body weight (r=0.35) for a diverse (age 19 to 65 years) group of 23 men and women. In summary, these studies generally support our finding that lean body mass provides the best body size index to predict blood volume.

Vascular fluid volumes are most accurately predicted through the use of regression equations and not by the use of simple ratios between volume and body size. Hurley (14) has developed regression equations to predict vascular fluid volumes from surface areas by surveying the published literature. As stated in the Introduction section, that database represented a very diverse popluation and employed measurements from many different laboratories. The present study's equations predicted erythrocyte volumes that are lower than those values predicted by Hurley's equations for a given body surface area. The greatest differences between the predicted erythrocyte volumes are for the larger subjects. The plasma volumes predicted by the present study's and Hurley's equations are nearly identical for a given body surface area.

The ratios of vascular volume to body size indices provide a convenient method to compare our results to those from previous investigations. A comparison of our data to previously published erythrocyte and plasma volumes for young men is provided in Table 2 (1,4,8,10,13,21,25,29,30). In order to obtain these values, we extracted relevant information concerning comparably aged male subjects from larger and more diverse databases and/or often make additional calculations (1,4,8,13,21,25,29,30). For erythrocyte volume, our values when expressed per unit surface area (1000 $ml \cdot m^{-2}$) are nearly identical to values from other studies that used ^{51}Cr methodology as mean values ranged from 834 to 1160 $ml \cdot m^{-2}$; however, the two earlier

studies (4,8) that used other radioactive tagging material (^{59}Fe and ^{32}P) found slightly higher values. The volumes of distribution for erythrocytes tagged with ^{51}Cr, ^{59}Fe and ^{32}P are believed to be similar (18) so the reasons for these small differences in erythrocyte volume are unclear. For plasma volume, our values when expressed per unit surface area (1685 $ml \cdot m^{-2}$) are similar to mean values reported by other studies. Together, these data indicate that the blood volume of healthy physically active young adult men approximates 2800 $ml \cdot m^{-2}$.

Intense physical training has been reported to acutely increase the plasma volume (7) and perhaps the erythrocyte volume (5,9) of young men. The question of whether these acute hematological adaptations persist and result in chronically elevated blood volumes for aerobically fit individuals who are not participating in a high intensity physical training program has not been previously addressed. Our data indicate that aerobic fitness does not correlate with either the erythrocyte or plasma volume of our subjects. Although significant relationships were found between aerobic power and blood volume per unit body weight, these relationships disappeared when corrections were made for lean body mass; therefore one can conclude that highly fit individuals might give the appearance of having a disproportionatly large blood volume because they have a relatively large lean body mass. Our data, however, should not be construed as being contradictory to previous observations that plasma volume will transiently expand during periods of intense exercise training and/or heat exposure (7).

Our study is the first to examine the effects of aerobic fitness on F-Cell ratio. The F-Cell ratio provides information concerning the erythrocyte distribution within the vascular space. Due to the Magnus effect there is a greater concentration of erythrocytes towards the blood vessels axis which results in "plasma skimming" or the flow of blood which is erythrocyte poor into the

branching vessels. This results in the hematocrit being lower in the smaller than larger blood vessels. Since skeletal muscle capillary density is increased with endurance training (12), we theorized that more aerobically fit persons might have a lower F-Cell ratio than less fit persons. We did not find any significant relationships between F-Cell ratio and aerobic fitness. Our subjects had an F-Cell ratio of 0.89 which compares favorably to values that we calculated from the data of the two other studies (4,8) which performed simultaneous measurements of erythrocyte and plasma volume in young men.

Our finding concerning the erythrocyte, plasma and blood volumes of young men are summarized as follows: 1) these vascular fluid volumes are accurately predicted from several indices of body size; 2) lean body mass is the anthropometric index which is most closely correlated to these vascular fluid volumes; 3) aerobic fitness does not influence these vascular fluid volumes in individuals not recently participating in an intense physical training; and 4) F-Cell ratio is not related to aerobic fitness. The normative database presented for erythrocyte volume, plasma volume and blood volume should have great value to clinicians and medical scientists.

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FIGURE LEGENDS

FIGURE 1. Individual data for the relationships of erythrocyte, plasma and blood volume with body weight. The solid lines represent the best fit regression.

FIGURE 2. Individual data for the relationships of erythrocyte, plasma and blood volume with body surface area. The solid lines represent the best fit regression.

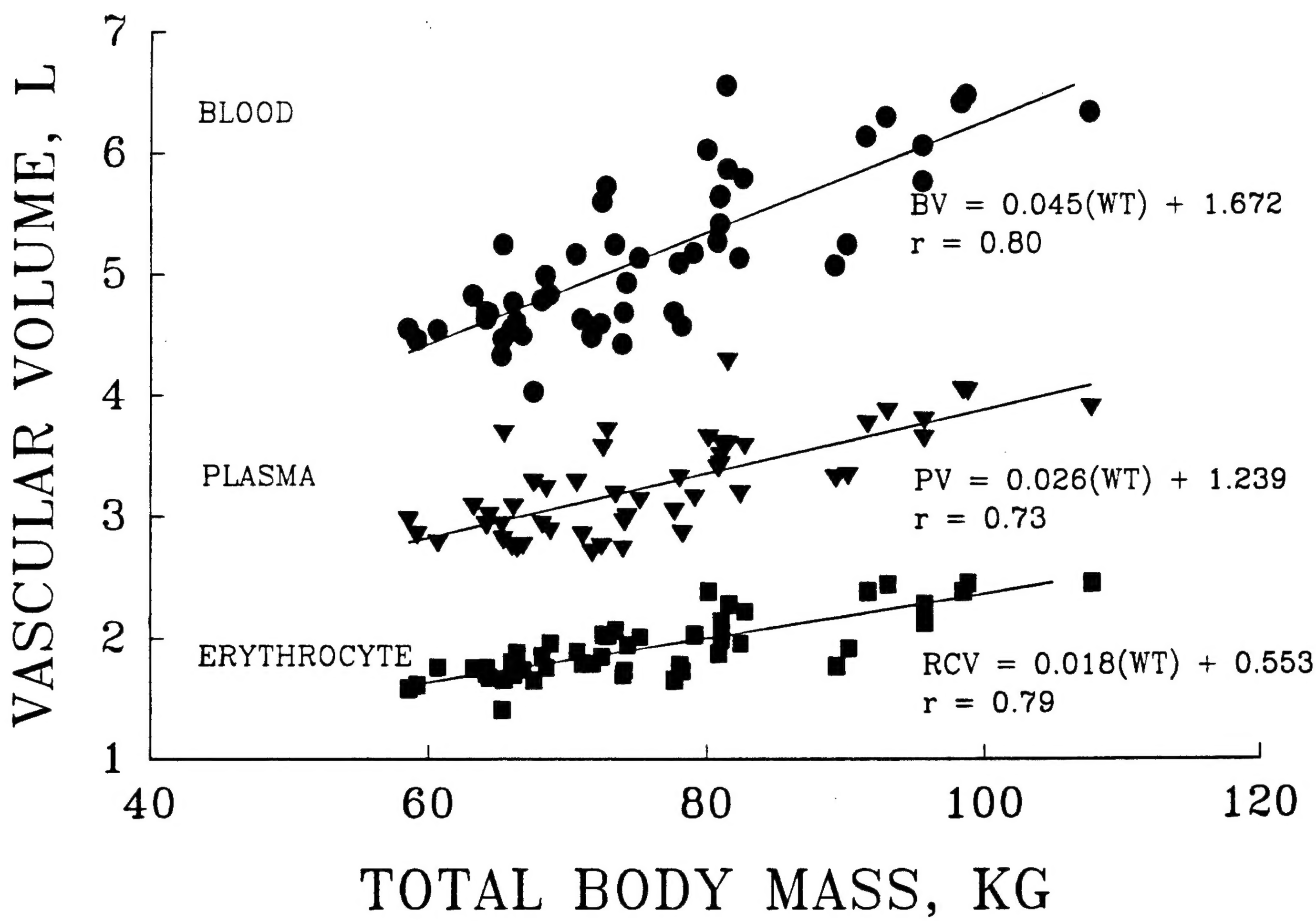
FIGURE 3. Individual data for the relationships of erythrocyte, plasma and blood volume with lean body mass. The solid lines represent the best fit regression.

Table 1. Characteristics of the population (n = 51) of male subjects.

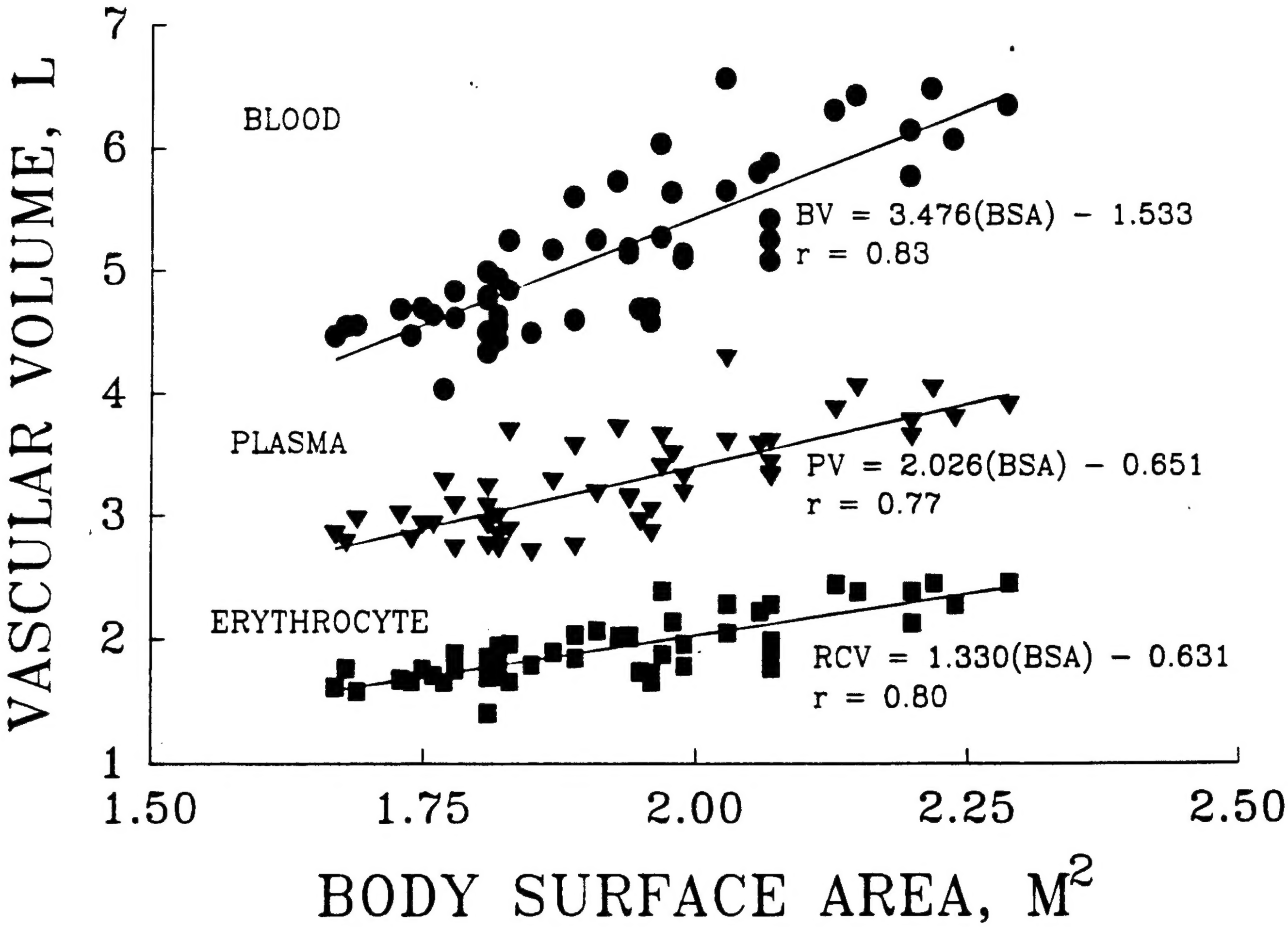
	Mean	Standard Deviation	Minimum	Maximum
Age (yrs)	22.08	4.95	18.00	35.00
Weight (kg)	76.08	11.42	58.59	107.91
Lean Body Mass (kg)	63.13	7.80	47.97	79.60
Body Surface Area (m^2)	1.92	0.16	1.67	2.29
Maximal Oxygen Uptake ($ml \cdot kg^{-1} \cdot min^{-1}$)	53.45	5.71	41.90	65.40

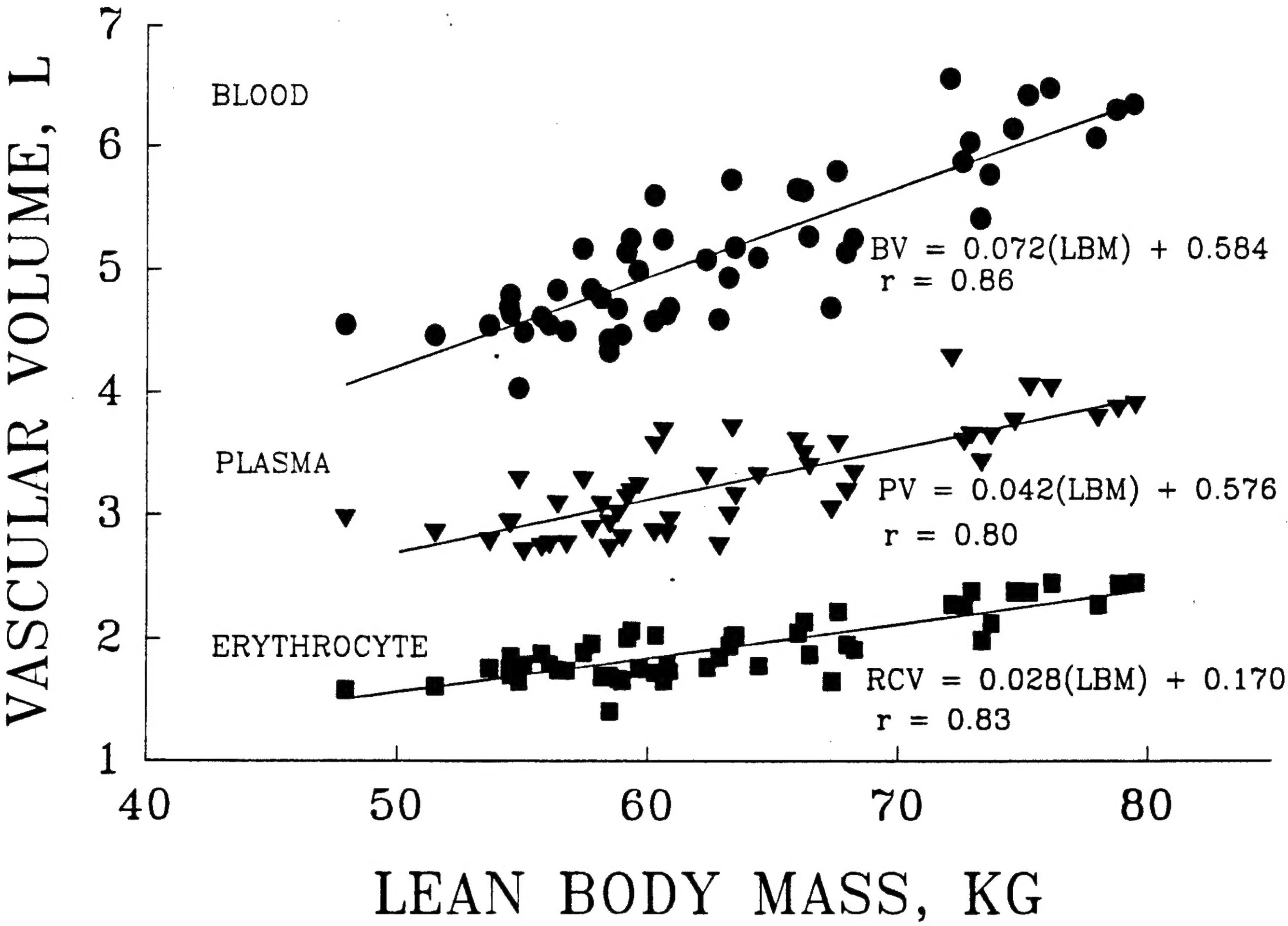
Chronological review of erythrocyte, plasma and blood volumes for adult men (16 to 35 years).

Study	Subjects	Method	Erythrocyte			Plasma			Blood		
			ml/kg	ml/m^2	ml/kgLBM	ml/kg	ml/m^2	ml/kgLBM	ml/kg	ml/m^2	ml/kgLBM
Gibson etal. 1946	40 (medical students)	T-1824 ^{59}Fe	30	1150	-	48	1849	-	78	2999	-
Steinbeck 1950	29 (medical students)	T-1824	-	-	-	47	-	-	-	-	-
Gregerson & Nickerson 1950	83	T-1824	-	-	-	46	-	-	-	-	-
Brady etal. 1953	20 (hospital patients)	^{131}I ^{32}P	32	1231	-	45	1731	-	77	2962	-
Allen etal. 1956	41 (Chinese students)	T-1824	-	-	-	49	1653	59	-	-	-
Huff & Feller 1956	33	^{51}Cr	28	1160	38	-	-	-	-	-	-
Muldowney 1957	10	T-1824	-	-	-	43	1685	52	-	-	-
Wennusland 1959	172 (prisoners)	^{51}Cr	28	1089	-	-	-	-	-	-	-
Vyas etal. 1965	89 (Indian patients)	^{51}Cr	25	834	-	-	-	-	-	-	-
Present Study	51 (soldiers)	^{125}I ^{51}Cr	26	1000	31	43	1685	52	69	2685	82



VASCULAR VOLUME, L
1
2
3
4
5
6
7
BLOOD
BV = 0.045(WT) + 1.672
r = 0.80
PLASMA
PV = 0.026(WT) + 1.239
r = 0.73
ERYTHROCYTE
RCV = 0.018(WT) + 0.553
r = 0.79
40
60
80
100
120
TOTAL BODY MASS, KG


VASCULAR VOLUME, L
BLOOD
BV = 3.476(BSA) - 1.533
r = 0.83
PLASMA
PV = 2.026(BSA) - 0.651
r = 0.77
ERYTHROCYTE
RCV = 1.330(BSA) - 0.631
r = 0.80
1
2
3
4
5
6
7
1.50
1.75
2.00
2.25
2.50
BODY SURFACE AREA, M2



VASCULAR VOLUME, L
7
6
5
4
3
2
1
BLOOD
BV = 0.072(LBM) + 0.584
r = 0.86
PLASMA
PV = 0.042(LBM) + 0.576
r = 0.80
ERYTHROCYTE
RCV = 0.028(LBM) + 0.170
r = 0.83
40
50
60
70
80
LEAN BODY MASS, KG